# Response to autoimmune enteropathy to cyclosporin A therapy

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## Abstract

Small bowel enteropathies that are associated with an autoimmune process are often resistant to treatment. Two children with autoimmune enteropathy were treated with cyclosporin A for eight months. Both improved, as assessed by growth, small intestinal mucosal morphology, and carbohydrate absorption. Cyclosporin A is useful in the treatment of autoimmune enteropathy. This report also suggests that T cell activation (which is suppressed by cyclosporin A) is important in the pathogenesis of this condition.

A proportion of children presenting with chronic diarrhoea and small intestinal enteropathy suffer from an autoimmune process. In one series, enterocyte autoantibodies were found in over 50% of infants with intractable diarrhoea.[1] The criteria for the diagnosis of an autoimmune enteropathy have been defined as follows:[2]

(a) Presentation with chronic diarrhoea and a severe small intestinal enteropathy;

(b) No response to exclusion diets;

(c) Evidence of predisposition to autoimmune disease (presence of circulating gut autoantibodies or associated autoimmune disease, or both);

(d) Immunocompetence.

The treatment of children with this condition remains unsatisfactory and a number of children have died.[1]

Activation of T lymphocytes is now believed to be the mechanism by which many organ specific autoimmune diseases are mediated.[3] Cyclosporin A has been widely used as an immuosuppressive agent in autoimmune diseases.[4]

Activation of T cells produces a severe enteropathy in an in vitro fetal intestinal organ culture.[5] These changes could be prevented by cyclosporin A treatment and this provided a rational basis for its use in two children with an autoimmune enteropathy.

## Methods

### PATIENTS

Two children with an autoimmune enteropathy were chosen for treatment with cyclosporin A. Their early clinical history has been described previously[1 2 6] and they satisfy the criteria for the diagnosis of autoimmune enteropathy listed above.

Immediately before the start of cyclosporin A treatment, proximal small intestinal biopsy specimens from both patient 1 (a boy aged 9·5 years) and patient 2 (a girl aged 12·3 years)

TABLE I  *Autoantibodies found in two patients with autoimmune enteropathy*

| Cell or organelle | |
| --- | --- |
| Patient 1: | |
| Enterocyte brush border | + |
| Pancreatic islet cells (type 1) | + |
| Renal cell brush border | + + |
| Mitochondria | + + |
| Patient 2: | |
| Enterocyte brush border | + |
| Pancreatic islet cells (type 2) | + |
| Nucleus | + |
| Smooth muscle | + |

showed enteropathy. Circulating autoantibodies were found against other organs as well as against gut (Table I).

Patient 1 had developed an interstitial nephritis and reduced glomerular filtration rate. A glucose tolerance test had also shown that he was prediabetic. Haemoglobin $A_{1c}$ concentrations were, however, normal.

Patient 2 had clinical diabetes and required regular insulin treatment. She also had severe juvenile chronic rheumatoid arthritis.

### CYCLOSPORIN A ADMINISTRATION

The effect of cyclosporin A treatment was assessed over eight months. It was initially given at a dose of 100 mg/m²/day in two divided doses, with the intention of achieving a circulating concentration of 50 ng/ml 10 hours after the dose. Because cyclosporin A was absorbed poorly, higher doses (430 mg/m²/day in each child) were needed to achieve these values.

Both children were receiving dietary supplements when cyclosporin A was begun. These included vitamin preparations (Ketovite), 1α tachysterol, and vitamin E. They were also prescribed bronchodilators, which they took infrequently for asthma. In addition, patient 2 had been receiving low dose corticosteroids (Deflazacort, 6 mg daily) and ibuprofen for arthritis. The dosages of these anti-inflammatory agents were not changed during cyclosporin A treatment.

Both children also received a diet that was free of milk and gluten.

### ASSESSMENT

Three groups of parameters were used to assess objectively the efficacy of cyclosporin A (subjective feelings of clinical well being were also noted):

### Growth

Both children were weighed before and after eight months' treatment. Height velocity was

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determined at the same times by taking the height gained over six months before treatment and that gained during cyclosporin A treatment. Height was measured using a stadiometer.

## Small bowel morphology

Small intestinal biopsy specimens were taken by a double port paediatric Crosby capsule[7] before beginning cyclosporin A and after eight months of treatment. Specimens from one port were fixed in formal saline, embedded in paraffin wax, sectioned, and stained with haematoxylin and eosin. They were examined under light microscopy. Adjacent specimens were fixed in glutaraldehyde for electron microscopy. Specimens from the second port were snap frozen in liquid nitrogen for immunochemistry.

TABLE II    *Effects of cyclosporin A on growth*

|  | Patient 1 | | Patient 2 | |
|---|---|---|---|---|
|  | Before treatment | After treatment | Before treatment | After treatment |
| Age (years) | 9·51 | 10·15 | 12·26 | 12·9 |
| Weight (kg) | 27·9 | 30·7 | 22·44 | 27·0 |
| Height (cm) | 118·5 | 124·9 | 131·6 | 137·6 |
| Height velocity (cm/year) | 6·45 | 10·29 | 4·34 | 9·08 |
| Height velocity SD score | +1·54 | +6·25 | −1·12 | +1·76 |
| Pubertal stage | 1 | 1 | 1 | 1 |

## Small bowel immunochemistry

Indirect immunoperoxidase was used to study intraepithelial lymphocytes in frozen sections because there is no endogenous peroxidase in the epithelium. Acetone fixed cryostat sections (8 μm) were incubated in primary monoclonal antibody followed by rabbit anti-mouse peroxidase conjugate, and reactivity was visualised using diamino benzidine as described elsewhere.[8] Primary monoclonal antibodies studied were CD3 (Dako UK Ltd) and TCR δ-1 (T Cell Sciences), which recognises the δ chain of the T cell receptor.

The alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was used to study cells in the lamina propria. This was used because there is no endogenous enzyme in lamina propria cells. Immunoperoxidase was used to study intraepithelial lymphocytes because of background staining in the epithelium seen with APAAP due to brush border alkaline phosphatase activity. Acetone fixed cryostat sections (8 μm) were incubated in primary monoclonal antibody, then in rabbit anti-mouse immunoglobulin followed by mouse APAAP. The last two layers were repeated twice. Reactivity was visualised using the fast red reagent.[9] Monoclonal antibodies used were CD3 and CD25 (anti-interleukin 2 receptor, Becton Dickenson).

Quantitation of lymphocytes was done using a ×40 objective. Intraepithelial lymphocyte frequency was determined by counting the number of peroxidase stained and unstained cells in the epithelium, and the number of stained cells was expressed as a percentage of the total. The frequency of lamina propria CD3+ and CD25+ cells was determined by counting the number of stained and unstained cells in the lamina propria, and the number of stained cells was expressed as a percentage of the total.
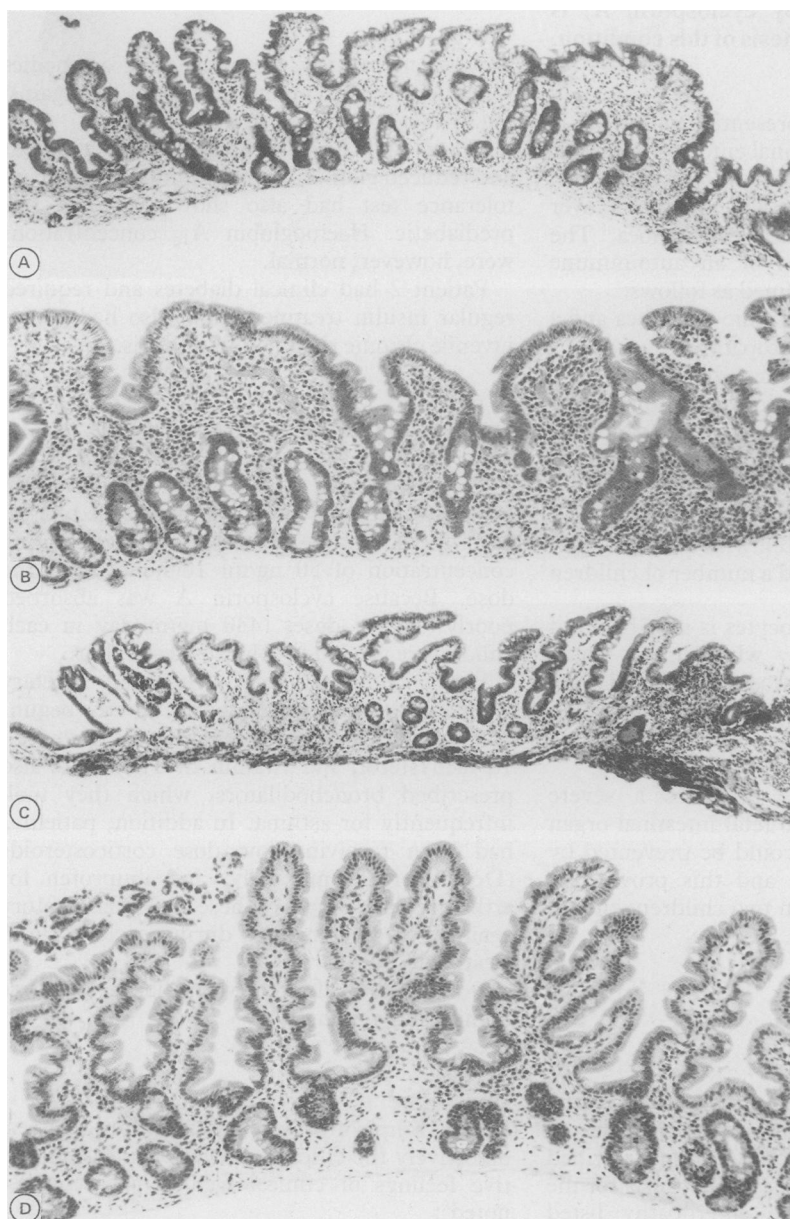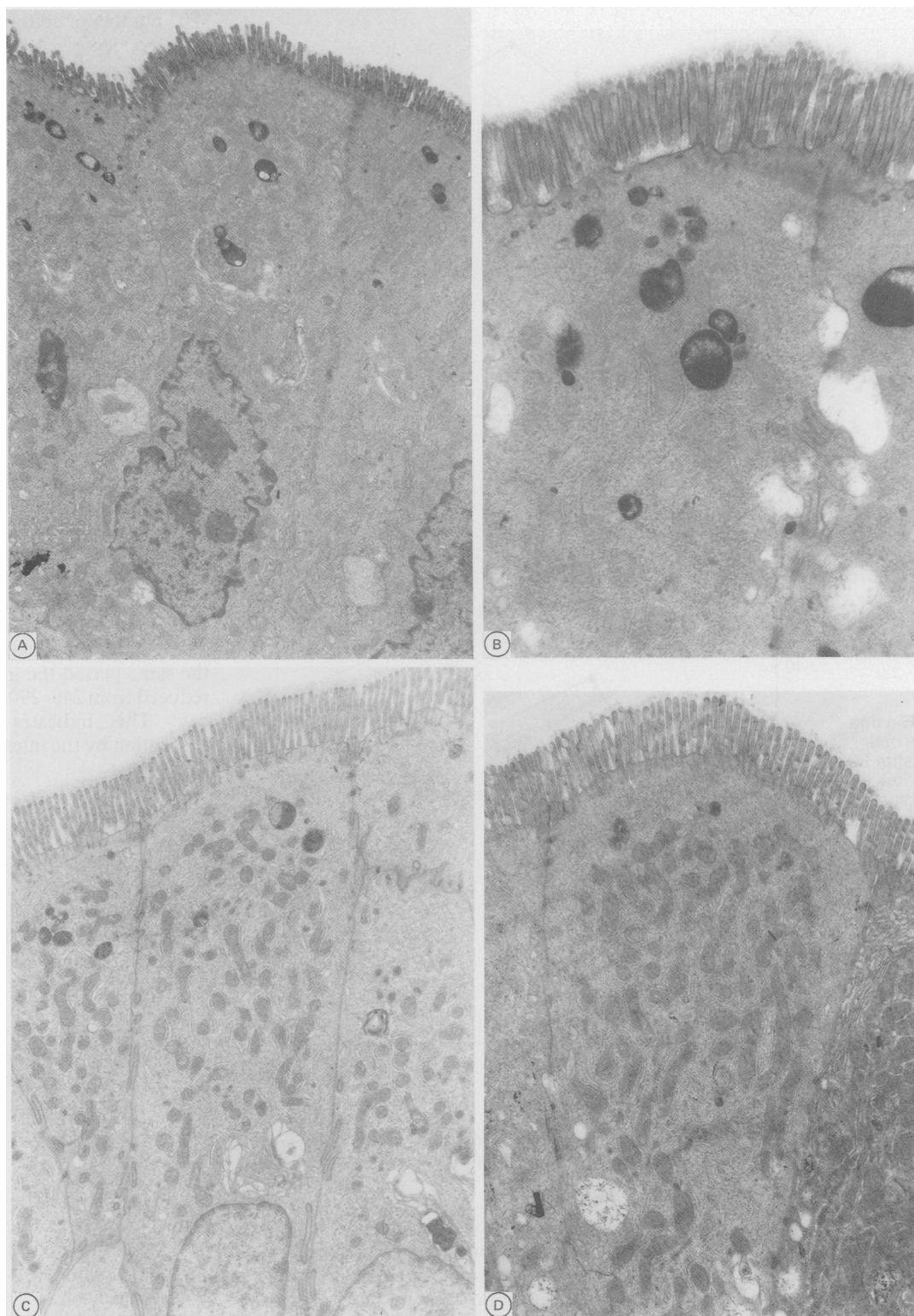


*Figure 1: Light microscopy of proximal small intestine. (A) Patient 1 before cyclosporin A treatment showing partial villous atrophy with crypt hyperplasia. (Original magnification ×80.) (B) Patient 2 before cyclosporin A treatment showing partial villous atrophy, crypt hyperplasia, and increased lamina propria cellularity. (Original magnification ×165.) (C) Patient 1 after cyclosporin A treatment showing no noticeable improvement over (A). (Original magnification ×80.) (D) Patient 2 after cyclosporin A treatment showing normal small intestinal mucosa. (Original magnification ×165.)*

## Carbohydrate absorption

The suboptimal production of insulin in these children afforded an opportunity to assess the absorption of carbohydrate by the small intestine. Patient 1 had prediabetes as shown by an abnormal glucose tolerance test after an oral load. Improved glucose absorption by the small intestine would lead to an increase in the blood glucose concentrations after an oral glucose load, provided that the insulin response to the load was unchanged. Patient 2 had clinical diabetes mellitus and it was considered inappropriate to administer a glucose load without insulin treat-

*Figure 2: Electron microscopy of proximal small intestine. (A) Patient 1 before cyclosporin A treatment showing columnar epithelium with short irregular microvilli and increased numbers of secondary lysozomes. (Original magnification ×11 500.) (B) Patient 2 before cyclosporin A treatment showing increased secondary lysozomes in columnar epithelium. (Original magnification ×23 000.) (C) Patient 1 after cyclosporin A treatment showing tall columnar epithelium of normal appearance. (Original magnification ×11 500.) (D) Patient 2 after cyclosporin A treatment showing tall columnar epithelium of normal appearance (Original magnification ×13 800.)*

ment. Her insulin requirements were, however, monitored while on treatment.

## ADVERSE EFFECTS

The children were examined clinically at least every three weeks during treatment. Blood pressure was measured and serum creatinine values assayed. As patient 1 had poor renal function before beginning therapy, glomerular filtration rates were measured directly using $Cr^{51}$-EDTA excretion, before and after eight months of therapy.

## Results

The effects of cyclosporin A were beneficial in the two children with autoimmune enteropathy.

## GROWTH

The effects of cyclosporin A on growth are shown in Table II. The change in the height velocity SD score is noticeably positive; and this cannot be attributed to acceleration caused by puberty as both children were prepubertal at the end of the assessment period. Both patients also gained weight.
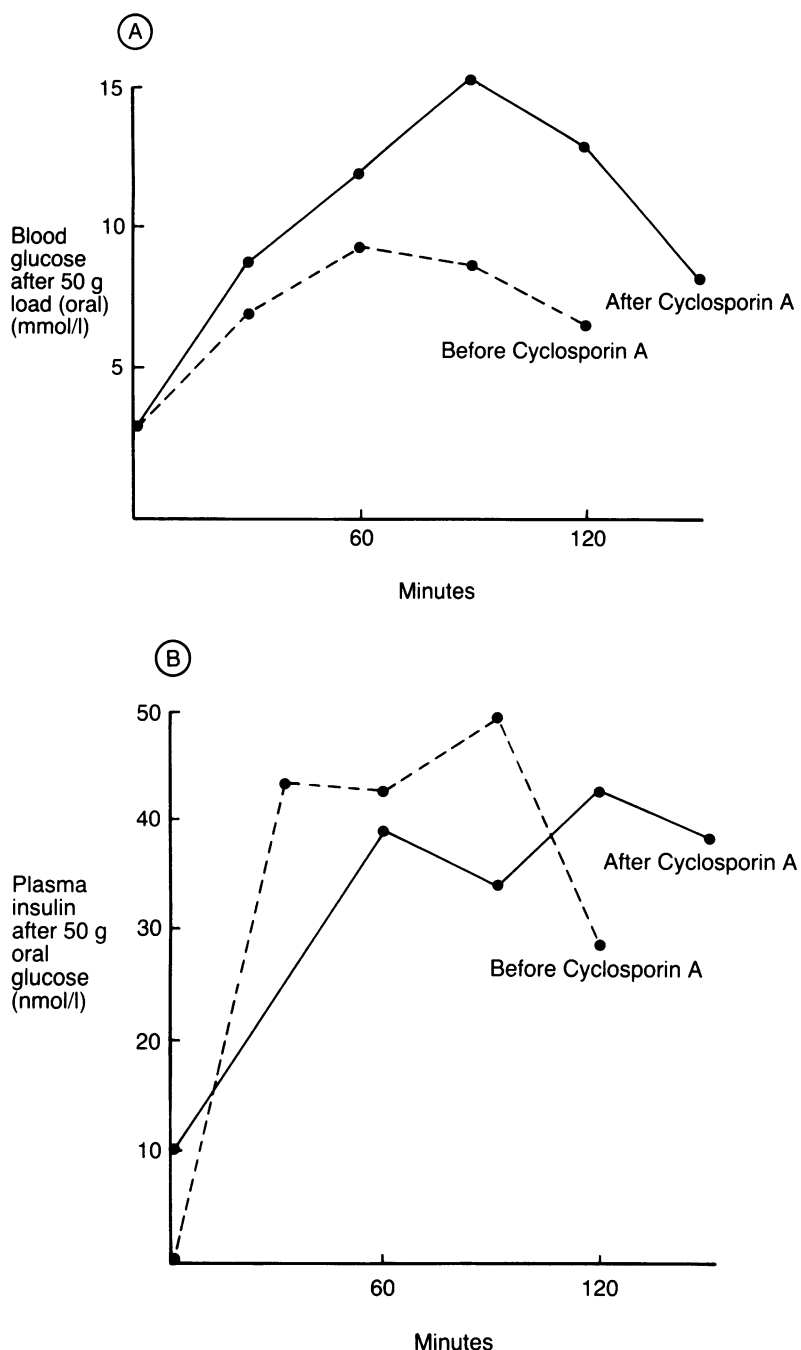
Figure 3: (A) Increase in blood glucose concentrations with 50 g oral glucose given after a 12 hour fast. (B) Increase in blood insulin concentrations with 50 g oral glucose given after a 12 hour fast.

SMALL BOWEL IMMUNOCHEMISTRY

The number of epithelial T cells fell appreciably with cyclosporin A treatment (Table III). There was a relatively greater reduction in those cells that expressed the γδ T cell receptor, though because of the low numbers of cells expressing the T cell receptor this may not be significant.

In the lamina propria, the number of nucleated cells expressing CD3 fell only in patient 2; however, there was a fall in the percentage of cells expressing CD25 in both patients.

CARBOHYDRATE ABSORPTION

Figure 3 shows the effects of cyclosporin A on the two hour blood glucose profile of patient 1. Glucose concentrations were greater after a 50 g oral load during cyclosporin A treatment. This figure also shows that there was no difference in the insulin response to the load (probably because this response was maximal on both occasions). This result can be explained by increased carbohydrate transfer by the intestine.

The insulin dosage needed to maintain glucose homeostasis in patient 2 (as assessed by regular home blood sugar monitoring) increased from 25 U daily (1·1 U/kg/day) to 52 U (1·9 U/kg/day) after 8 months' cyclosporin A therapy. During the same period the intake of carbohydrate was reduced from 240–290 g per day to 200–230 g per day. This indicates increased carbohydrate absorption by the intestine.

ADVERSE EFFECTS

There was no deterioration of glomerular function as shown by creatinine concentrations in patient 2. The glomerular filtration rate of patient 1 on entry to the study was 28 ml/min/ 1·73 m², and after eight months' cyclosporin A treatment it was 26 ml/min/1·73m². This change is not significant, as it is within the accuracy of the technique.

Although both children were noted to have developed increased hair on their limbs and over the back, this was not sufficient to cause concern to them or their parents.

SMALL BOWEL MORPHOLOGY

Patient 1 did not show an appreciable improvement in tissues examined by light microscopy (Fig 1) but electron microscopy showed that the ultrastructural appearance of the epithelium had reverted to normal (Fig 2). Patient 2 showed both light and electron microscopic improvement.

TABLE III   Number of cells staining for pan T-cell marker and the interleukin 2 receptor in the lamina propria and for pan T cell marker and γδ T cell receptor (TCR) in the epithelium in patients 1 and 2 before and after cyclosporin A treatment

| | Patient 1 | | Patient 2 | |
|---|---|---|---|---|
| | Before | After | Before | After |
| Lamina propria nucleated cells expressing CD3 (%) | 20·7 | 28·7 | 54·9 | 38·2 |
| Lamina propria nucleated cells expressing CD25 (%) | 8·2 | 3·3 | 11·4 | 2·9 |
| CD3/100 epithelial cells | 14·1 | 7·4 | 18·3 | 8·2 |
| TCR δ-1/100 epithelial cells | 1·0 | 0·2 | 1·4 | 0 |
| TCR δ-1/100 CD3 cells | 7·1 | 2·7 | 7·6 | 0 |

Discussion

This report clearly shows the benefit of cyclosporin A treatment in these two children with autoimmune enteropathy who had been unwell for many years. Not only was the treatment beneficial, as shown by its effect on intestinal structure and function, but it also led to an acceleration of linear growth that was not related to puberty.

Cyclosporin A suspends certain nuclear events associated with T cell activation.[10] Cytotoxic lymphocytes are inhibited from acquiring responsiveness to interleukin 2.[11] Interleukin 2 production is inhibited both in the primary response to antigen[12] and in response to antigen restimulation.[13] Also interleukin 1 production of antigen presenting cells is curtailed.[14] In our patients, interleukin 2 receptor expression, a marker of T cell activation, was reduced within the lamina propria after treatment with cyclosporin A. T cell activation can cause an entero-

pathy in vitro;[5] and this report suggests an important role for T cells in the pathogenesis of autoimmune enteropathy. The observation[15 16] of aberrant expression of HLA-DR antigen in the enterocyte supports the concept of a central role of T cell activation in the pathogenesis of autoimmune enteropathy.

The importance of autoantibody in the pathogenesis of the mucosal damage has been questioned. Antibody concentrations do not necessarily correlate to the severity of disease. Indeed, circulating autoantibodies have not been detected in some patients at the time of presentation, but occurred at a later date. Interestingly, an autoimmune enteropathy has been described in a patient with common variable immunodeficiency in whom production of IgG and IgA class antibodies by plasma cells was defective.[17] These inconsistencies can now be resolved because T cell activation, which is the primary event in autoimmune disease, does not necessarily cause the organ damage by way of B cell activation and antibody production, which may be a secondary phenomenon. An example of an autoimmune disease where T cell induced damage is caused by a cellular mechanism is experimental autoimmune uveal retinitis seen in the rat.[18] In this condition, autoantibodies are produced against a retinal antigen, yet the disease is mediated by T cells. T cell activation can be prevented by using cyclosporin A, with consequent prevention of ocular inflammation, yet circulating autoantibodies are still produced.

We adopted a cautious approach to the amount of cyclosporin A prescribed. Interestingly, the circulating concentrations that were effective were lower than those commonly used in prevention of organ transplantation rejection. Adverse effects of the drug are therefore less likely. Indeed the only side effect noted was mild hirsutism. It is, however, impossible to predict the long term effect of this treatment.

In conclusion, cyclosporin A has been shown to be useful in the treatment of autoimmune enteropathy. Its efficacy suggests that activation of T cells are central to the pathogenesis of this condition.

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